Modeling the genetic dual-feedback oscillator

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***Short Abstract* — Recently, we constructed a genetic oscillator comprised of coupled positive and negative feedback loops. The robustness of the observed oscillations, however, challenged our understanding of the network, which was based on previous theoretical work. We find that a more complete model of the system not only quantitatively predicts the dynamics of the oscillator, but also changes our qualitative understanding of the mechanism behind oscillations. Introduction of several intermediate steps create a small delay in the feedback loops, leading to robust relaxation oscillations. Furthermore, our modeling predicts, and experiments confirm, that the system oscillates even in the absence of positive feedback.**

***Keywords* — Gene oscillator, gene regulation.**

I. Introduction

One defining goal of synthetic biology is the development of gene-regulatory networks according to "design specs" generated from computational modeling [1,2]. Such an approach provides a systematic framework for exploring how a given regulatory network generates a particular phenotypic behavior. This method has resulted in the development of several fundamental gene circuits, such as toggle switches [3] and oscillators [4,5]. Here we describe a computational model of the genetic dual feedback oscillator [6]. The oscillator was constructed in *E. Coli*, based on a previously modeled network architecture comprising linked positive and negative feedback loops [7].

After experimental characterization of the oscillator we found that the original computational model inaccurately describes its behavior and fails to account for the robustness of observed oscillations. These findings led us to a new computational model, which is still based on the concept of coupled positive and negative feedback loops, but includes many details of the system which were omitted from previous reduced models. We find that directly modeling protein-DNA binding, multimerization, DNA looping, enzymatic degradation, and protein folding greatly increases the accuracy of the model. Moreover, these additional steps qualitatively change the underlying mechanism of the oscillations by providing small but essential delays in the activity of transcriptional regulators.

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II. Results

Our modeling of this system led us to two important insights into the behavior of the present genetic oscillator. First, a "complete" model of the system predicts the behavior much better than a reduced model, obtained via a quasi-steady state approximation (QSSA). The reason for this lies not just in the time scales of the system, but also in the sequential timing of events. Because the intermediate steps in the formation of functional proteins take time, introducing them into the model creates an *ad hoc* form of delay. We find that this delay leads to robust oscillations with a period much greater than the delay time. Furthermore, the complete model correctly predicts the period of oscillations as a function of inducer concentrations.

Second, the presence of even small delays suggests that the oscillator may work in the absence of positive feedback. Delayed negative feedback is a known mechanism for oscillation [8], and our model oscillates weakly without the positive feedback loop. To test this prediction, we performed further experiments with our genetic oscillator, in a strain in which the positive feedback loop was disabled. As predicted, erratic, low amplitude oscillations were observed in this strain, consistent with the model.

III. Conclusion

Based on detailed computational modeling, we are able to explain the nature of the oscillations observed in the recently constructed dual-feedback gene oscillator. Sequential intermediate steps in transcriptional regulation provide delay in the activity of feedback loops and lead to robust relaxation oscillations. Our results provide new design principles upon which further synthetic circuits can be designed and tested.

References

[1] Hasty J, McMillen D & Collins JJ (2002) Engineered gene circuits. *Nature* **420**, 224-230.

[2] Endy D (2005) Foundations for engineering biology. *Nature* **438**, 449-453.

[3] Gardner TS, Cantor CR & Collins JJ (2000) Construction of a genetic toggle switch in *Escherichia Coli*. *Nature* **403**, 339-342.

[4] Elowitz MB & Leibler S (2000) A synthetic oscillatory network of transcriptional regulators. *Nature* **403**, 335-338.

[5] Fung E, et al. (2005) A synthetic gene-metabolic oscillator. *Nature* **435**, 118-112.

[6] Stricker J, et al. (submitted) A fast, robust, and tunable synthetic gene oscillator. *Nature*.

[7] Hasty J, et al. (2002) Synthetic gene network for entraining and amplifying cellular oscillations. *Phys Rev Lett* **88**, 148101.

[8] Bratsun D, et al. (2005) Delay-induced stochastic oscillations in gene regulation. *PNAS* **102**, 14593-14598.